

Water And Alcohol Extraction Of Thyme Plant (*Thymus Vulgaris*) And Activity Study Against Bacteria, Tumors And Used As Anti-Oxidant In Margarine

Manufacture

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Abstract:

Isolation had been done for active substances from Thyme plant (*Thymus Vulgaris*) such as volatile oils, Saponins and Tannins. The percentage in form was (21.1%),(59.2%),(9.7%) respectively. Also a study of anti-bacterial activity of extracts from Thyme using two types of pathogenic bacteria *Escherichia Coli* and aurous *Staphylococcus* showed the ability of inhibition for all different extracts by vary inhibition diameters for different active substances, concentrations and bacteria. One type of cancer cellular line used to study the effect of Thyme extracts on the growth of cells in the laboratory and thus know the specifications of extracts as anti-tumor, (L₂₀B) cell line have been used which is mice Transformed cell Line. The possibility of using alcohol extract as anti-oxidant to be add to ghee or vegetable oil, the sensory evaluation and physical results were very good over full year.

Keywords: *Thymus Vulgaris*, Antibacterial and anticancer activity. Extraction, Anti-Oxidant

Introduction:

The medicinal plants are important source effective materials, intervention in the preparation of many drugs. It has been proven scientifically that active laboratory manufacturer substance does not perform the same physiological influence role of active derived substance from medicinal plants, also in addition side effects of prepared material on the body, which may not appear until after a period may be long, Thyme plants is the well-known species of oral and scientific name (*Thymus Vulgaris*) [1].

Thyme plants is the well-known species of oral and scientific name (*Thymus Vulgaris*) [1]. *Thymus vulgaris* or common thyme is a low growing herbaceous plant, sometimes becoming somewhat woody. It is native to southern Europe, where it is often cultivated as a culinary herb. It typically grows as a sub-shrub, between 15 and 20 cm tall. [1]. *Thymus vulgaris* Figure (1) is a commonly used culinary herb and a basic ingredient in French, Greek, Italian, Lebanese, Persian, Portuguese, Spanish, Syrian, and Turkish cuisines.



Figure (1) *Thymus vulgaris*

Thyme frequently in Mediterranean Sea basin, it has strong aromatic smell and classified from the scope of eukaryotes flowering plants division, binaries monocots range, oral platoon rank. Thyme is shrub perennial superior to 12-inch, small leaves sprout from stem, pink or purple flowers with unique characteristic odor, a little bit hot taste growing over most moderate climate areas. It is used in folk medicine to treat many diseases including respiratory disease (cough pertussis, bronchitis and asthma) and has analgesic properties, antiseptic, stimulant for the circulatory system, facilitates the secretion of sweat, urine generates, heart muscle tonic, prevents hardening of the arteries, treated urinary tract infection and reduce cholesterol [2]. Thyme also stimulates the memory, treatment of gums, dental pain, mouth inflammation, larynx, trachea and enters in the manufacture of toothpaste because it is perfumed the mouth, Thyme works as stomach alert, gases expulsion, prevent fermentation, helps the digestion, nutrients absorption, expel fungus from stomach and intestines, increase appetite for food because it contains Thymol which kills bacteria and parasites from stomach, in addition Rvkrol which is an analgesic, antiseptic, expectorant, anti-bleeding, diarrhea and anthelmintic. Experience proven that Thyme oil kills amoeba cause dsntrella, lebed colon bacteria and increases body weight because it helps digestion and absorption of fatty acids. Thyme oil (common as Thymus) with a pungent odor and medical benefit has more than 44% of phenols, which mainly consists 41% of Thymol and 3.6% Karvacrol, as confirmed by studies oil contain Polyphenolic acids which are Caffèic acid, Triterpene, Rosmaric acid and Oleanic acid, while resins, gums and tannins are about 10% of the components of this plant, and as a result of an anti-bacterial properties it used as a disinfectant which is the main active ingredient in Listerine and toothpaste. Before modern antibiotics, it was used in dressings because it active against fungus, foot toenails and generally is used for cough and bronchitis. Medically, Thyme is used for respiratory infections in form of tisane, ointment, syrup and steam inhalation. Thyme if boiled in water and cold it is very effective against larynx inflammation and gargling 3 times a day. Inflammation will disappear usually within 2 to 5 days. Injuries and other wounds can be distilled from the boiling. After birth mother gives Thyme tea. so oxytocin makes semi-deflationary effect of the uterus, improving digestion and relaxes smooth muscle and reduces prostaglandin which causes contractions of the muscles and that will benefit of athletes and eliminate intestinal parasites. Oil also contain gelation materials, antiseptic and pain eliminating contain sugars without alkaloids. Thyme oil also contain other substances (Borneol, menthone, pinene, cymen, Pinen, Sabinen, Gerniol, Limonen, Myrcen, Cineol and linalool). It is strong anti-oxidant because of its content higher phenolic acids and flavonoids, the most important Eridicytol, Luteolin, and Apigenin [3], and also Thyme contain in it structure

ineffective materials saturated fatty carbohydrates, proteins, vitamins (A, B, C), iron and manganese elements, and high concentrations of chromium and nickel elements, fiber and widely use Thyme in all over the world [4].

Methods:

First: Source and classification of plant:

Thyme Used in this research was obtained from the local market, imported from Syria.

Second: Materials and methods of work for water and alcohol extraction:

Thyme (*Thymus Vulgaris*) were grounded and kept at a laboratory temperature until use, for the preparation of aqueous extract (40) grams of Thyme powder were taken, placed in the conical flask containing (200) cm³ of distilled water, mixed by magnetic blender for (30) minutes and a centrifuge for (15) minutes. After that the solution stand in the electric furnace at (35) °C until we get the extract and from it we prepared solutions of (25, 20, 15, 10, 5)%.

Alcohol extracted obtained from putting (50) g of Thyme powder in a extraction unit (Soxhelt) and added (350) ml of ethanol (80%), extraction continued for (12) hours at (40) °C by using Vacuum Rotary Evaporator, also at (35) °C [4] after that by the same way aqueous extract solutions prepared [5].

Third: Active competent isolation:

A: Tannins:

Tannins was isolated from Thyme by adding (75) ml of distilled water to (0.5) g of Thyme powder The mixture put in boiled water bath for (30) minutes, then the mixture run in centrifuge at (200 cycle \ minutes) for a period of (20) minutes .The solution transfer to flask (100) ml and complete the volume to the mark with distilled water then added to the mixture (20) ml of 4% lead acetate with shaking then continued and filtered. The sludge dried at (70) °C in electric furnace [6].

B: Saponins:

Weight (10) g of Thyme powder added to (50) ml of (20% ethanol) and then heated using a water bath for half an hour and (55) °C with constant stirring, after that the solution filtered and separated then added to it (100) ml. The solution heated by using a water bath at (90) °C until final solution volume become (40) ml, where the filtrate transfer and added to it (20) ml of ether in separation funnel then water layer separated and ether layer neglected, add to water layer (10) ml of n-butanol then the resulting solution evaporation in water bath and dry the solution to get saponins [7.8].

C: Volatile oil:

Volatile oils extracted in Thyme by continues extraction method by using (Soxhlet) device, then by using ether as organic solvent where (5) g of Thyme powder with (150) ml of ether were carried out by extraction process for a period of (24) hours after that we separate solvent from volatile oils [9].

D: Chemical study (qualitative detection):

For the purpose of identification of Thyme components several chemical qualitative detections have been done for the initial extract of Thyme, as follows:

Semi-alkaloids detection, carbohydrates detection, Saponin [10], Flavonoids [10]:
Lipids [11] , Protein, and Tannins[10-11].

Fourth: Study the activity of anti- pathogenesis bacteria:

Agar-well diffusion method followed as the using of kirby Baaue method [15] in the measurement of the sensitivity of bacteria used in this research for various concentrations of substances derived from Thyme, Escherichia Coli and aurous Staphylococcus bacteria was obtained (isolated and diagnosed in culture laboratory in children's hospital in Ramadi). also we used Mueller Hinton ager to test the sensitivity of bacteria for Thyme extracts and it was prepared as company instruction process, then the dishes putted in incubator at (37) °C for (24) hours and inhibition diameter was then measure (Inhibition Zone) [15] in each hole by ruler and record the results.

Preparation of standard solutions of isolated substances from Thyme:

A series of different extracts solutions have been prepared for concentrations of (5%, 10%, 15% 20% 25%) mg / ml.

Test the effect of extracts against cancer cells:

One type of cancer cell lines have been used to study the impact of the extracts under study for Thyme extracts on the growth of cells in laboratory and thus know the specifications of extracts as an anti-tumors, this work done at Department of Cancer Research in Bio-technology Research Center, University of Nahrain.

Cell line used is (L₂₀B) which is a mice transformed cell line. In this way the proportion number of cells calculated within the optimal conditions for growth without the addition of extracts in and then the output is the control group (control). Then extracts are added for the purpose of knowing their effects on cell growth in the elected lines.

Use of Thyme as anti-oxidant in the manufacture of margarine:

Warm alcohol extract of Thyme used, which contains the highest percentage of volatile oils, flavonoids and phenols that consists mainly from Thymol and carvacrol on oil and margarine. The samples tested by adding extract at 100 mg per kg of margarine under test which is not additive by industrial additives were the physical and sensory changes have been studying by control the product for a full year and record all the notes of the color, taste and smell to evaluate the results.

Result and discussion

Table (1) and Scheme (1) shows the percentage of active contents that has been isolated from Thyme (Thymus Vulgaris) and the percentage of Tannins, Saponins and volatile oils are (9.2%,23.1%,50.7%) respectively, where it is noted that the volatile oils has recorded the highest percentage by weight in the Thyme, followed by saponins and Tannins.

This study showed qualitative testes of active compounds contained in Thyme and the presence of flavonoids, carbohydrates, condensed tannins, catechol, loquanthucyandin, saponins and phenolic acids.

Tables (2,3) shows a the anti-bacterial activity results of Thyme extracts. The activity have been studying for these extracts separately, in different concentrations and using two types of pathogenic bacteria Escherichia Coli and aurous Staphylococcus. The water extracts of Thyme shown higher activity at (25) mg / ml, where inhibition diameter was (21) ml for Staphylococcus aurous and (15) mm for Escherichia Coli, followed by the rest of the varying concentrations and rates table (2).

The inhibition action of alcohol extract is due to it contained Thymol, flavonoids, tannins, and include some of phenolic compounds which have a biological influence on many bacteria races due to the presence of hydroxyl groups (-OH), as it have the ability to form hydrogen bonds between hydroxyl group in these

compounds and water molecules in bacterial cell, where water is (90%) of weight and that will disable dynamic actions in bacterial cell [16]. These compounds as phenolic compounds have the ability to coagulate the bacterial cell proteins and destroy enzymes involved in the manufacture of necessary amino acids to increase cell division [17].

In general, from tables (2&3) for all extract and in all prepared concentrations the influence is almost equal against bacteria *Staphylococcus* and *Escherichia Coli*. It was found that the inhibition diameter for warm alcohol extract is bigger than for the rest of the extracts of other due to the varying rate of active materials in different extracts.

Table (1): Percentage of active components in Thyme (*Thymus Vulgaris*)

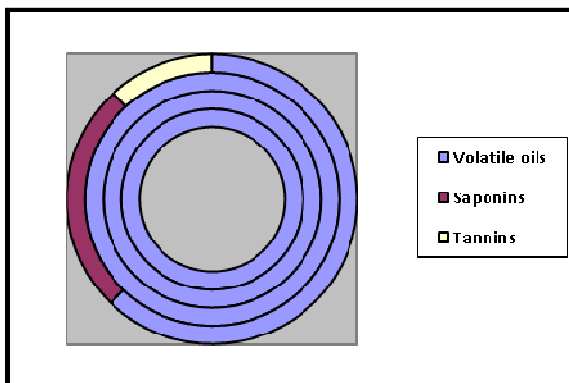
Active components	Percentage
Volatile oils	50.7%
Saponins	21.1%
Tannins	9.2%

Table (2): Effect of hot alcoholic extract of Thyme in different concentrations on growth of pathogenic bacterial strains

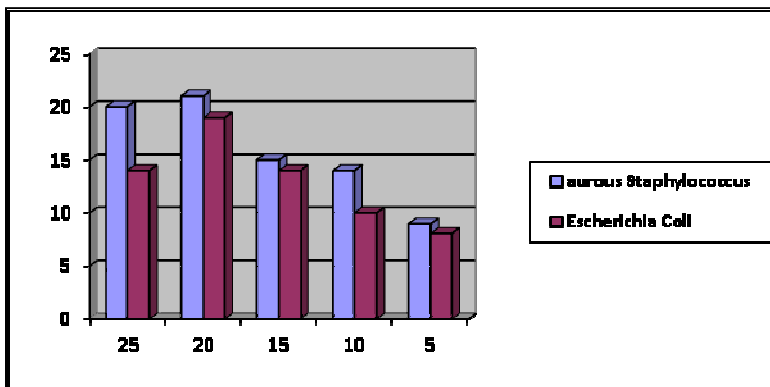
Conc. in mg/ml	Inhibition diameter	
	<i>Staphylococcus aureus</i>	<i>Escherichia Coli</i>
25	21	19
20	15	14
10	14	13
10	11	10
5	9	8

Table (3): Effect of hot water extract of Thyme in different concentrations on growth of pathogenesis bacterial races

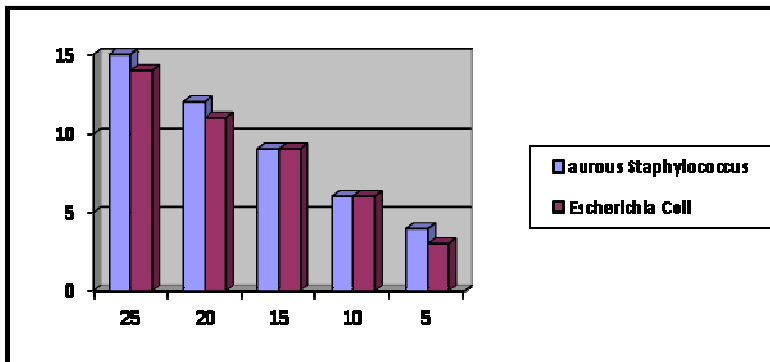
Conc. in mg\ml	Inhibition diameter	
	Staphylococcus aureus	Escherichia Coli
25	15	14
20	12	11
10	9	9
10	6	6
5	4	3



Scheme (1) Percentage of extracts materials



Scheme (2) Inhibition percentage of alcoholic extract of Thyme in different concentrations against pathogenic bacteria



Scheme (3) Inhibition percentage of water extract of Thyme in different concentrations against pathogenic bacteria

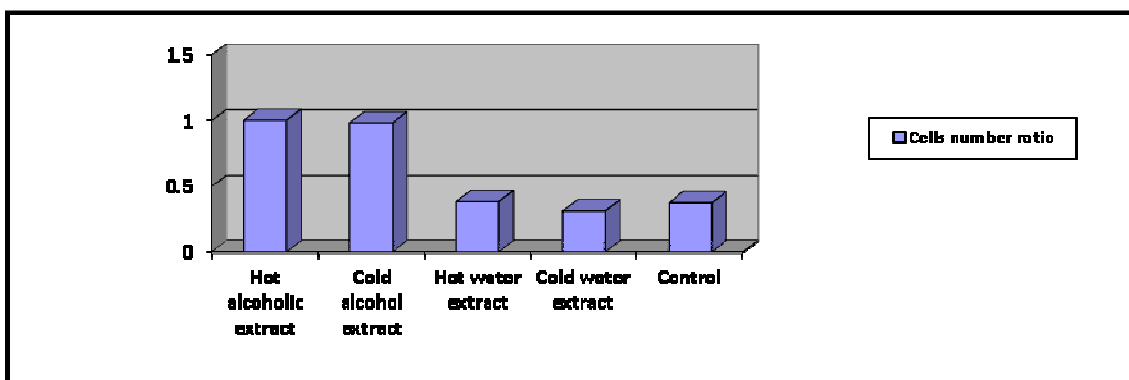
Test the effect of extracts against cancer cells:

Cancer cell lines have been used to study the effect of Thyme extracts on the growth of cells in laboratory to know the specifications of extracts as anti-tumors. cancer cell line type mice transformed cell line (L₂₀B) used in this study.

In this method, we calculate the proportion of cells number within the optimal conditions for growth without the addition of extracts so the output is the control group (control). Then extracts are added for the purpose of knowing their effects on cell growth in elected lines.

Extracts were divided into five groups, first group included cold water extract, second group included a hot water extract, third group cold alcohol extract, fourth included hot alcoholic extract, and fifth group is oil extract of Thyme.

The result statistically analyzed by one way ANOVA. the following results as scheme (4) which demonstrates the impact of compounds on cells number ratio when using cell line (L₂₀B), it is clear that hot alcoholic extract have the greatest influence on the proportion of growth cell number and the effect was significantly (P < 0.05). This result is identical to those published in literature [18-24]. Also the effect of aqueous extract was significant effect (P < 0.05) but the percentage of inhibition - as in the scheme - less effect than alcoholic extract. All other Extracts have inhibition effects on growth of transformed cells.



Scheme (4): effect of a range of compounds on the growth of cells in cell line (L₂₀B)

The use of alcoholic extract of Thyme as anti-oxidant in the manufacture of oils and margarine:

Of the most important chemical properties of vegetable oils is oil rancidification, which is a chemical change occurs a change in color, odor and taste of oils or fats, the reason is neither the oxidation process as a result of exposure to air, moisture and high temperature this will produce aldehydes, ketones, oxides and free radicals, or the process of bacteria decomposition where secreted enzymes will decomposed oils and fats to short chain and volatile fatty acids.

Anti-oxidants are used in foods which containing fats and oils in order to prevent oil rancidification that cause out an unpleasant odor from food product when it exposed to oxygen during storage, for example, anti-oxidants added to potato slices in order to prevent rancidification of the oils used in preparation, most of anti-oxidants used in the world are artificial, such as Butylatedhydroxyanisole (BHA), coded E320 and the other type Butylatedhydroxytoluene (BHT), coded E321. There are natural barriers such as vitamin E, vitamin C and ascorbic acid, it is preferably to use natural barriers than industrial materials [25_{a,b},26].

Generally we can say that use of food additives of natural origin (pigments, additives, anti-oxidants and local) there are no trap on them, but the global objection on use of industrial additives, especially if there is a natural alternative. In developed countries, government legislation sets the names of permit food additives for use. The law provides the names of these food additives on the packaging card, many laws issued that specified permitted nutritional additives for use, which are mandatory as standard European and American specifications. Several vitality and physiological tests done to make sure they are not harmful to human health and it is within the allowed concentrations, where addition most at the minimum possible amount to achieve intended purpose and are safe for consumption in the diet.

Additives materials : Cans food, widely packaged as many materials added to keep as long as possible for human consumption. So that addition done according to specified global standards without decrease or increase, because decrease lead to damage canned product while increase lead to countless diseases involve with poisoning [27].

Additive knows as substance added to food prevent any changes on qualities and it has an important role in preserving foods for long periods without damage or corruption and to reduce consumer exposure to poison or health harms as a result of keeping food in an unhealthy way. Anti-oxidants are the most important additives that work to prevent or delay chemical changes that occur as a result of interaction of oxygen with oil, fat, vitamins soluble in fat and essential fatty acids, which lead to rancidification where the food spoil and make it harmful for human health.

The additives are consider as safe and secure according to available scientific information for all members of society, except in cases that suffer sensitive to these additives which are rare, the specialists in the field of human health in European union countries specify the added concentration degree which is not show any negative effects and to increase the safety it have been allowed the use of up to 100 mg per kg of body weight, this level or low concentration called the acceptable daily intake, that represents the daily intake of human throughout, without damage the health. From above it can be said that the natural antibiotic mostly contain phenolic material and industrial which is derivative of phenolic. It is used more widely for its effectiveness and prevent the oxidation higher than normal, this addition is an important technological process in oils conservation and all foods contain oils to prevent emergence unpleasant flavors and smells that arise from rancidification and to prevent the appearance of oxidative products of oxidation, particularly polymers that carry a significant risk to human

health. For oil protection, it had been used unlimited number of global international authorized anti-oxidants. They are phenolic compounds with enough activity and don not cause any unpleasant color or flavor. The widely most important industrial anti-oxidant additives used in food are BHA, BHT and Pg. All used with vitamin C when they are used in food [28], unfortunately there are recent studies mention that these additives have a carcinogenic effect, and studies are continuing in this field [29-31].

It is worthy to mention that in 2007 the amount of 0.88 million tons of industrial anti-oxidants used and 3.7 billion dollar or the equivalent of 2.4 billion Euros, this demonstrates the massive amount and huge sums of money for this industry [32].

In this study, hot alcoholic extract of Thyme used in an attempt to produce new natural anti-oxidants can be used as a substitute for industrial inhibitors. From the experience on oil and pure plant fatty without industrial additions and following up physical and sensory color of taste and smell for a whole year we did not find through evaluation any specifications changes on oil and vegetable fat in the rate of 100 mg / kg which is within the allowable global rates. On other side, we found that alcoholic extract of Thyme and contents of flavonoids, phenolic compounds and volatile scented pure oils, as possible to give good flavor to oil as well as it function as an anti-oxidant, also it is possible to work as an alternative additives as flavors to food. From previous results of alcoholic extract importance against pathogenic bacteria and against cancer cells it could be added to give significance of used as natural anti-oxidant can be used as an alternative of industrial oxidation inhibitors.

In terms of match requirements for anti-oxidants materials, all international conditions available of quality and purity as a natural substance with 100% purity, and does not interact with the components of a food or their containers, this is what has been inferred from storage period for one year of this study, and do not effect on sensory and physical characteristics of the food material, this was obtained through follow-up to these qualities, and be able to spread in the food material without a catalyst, that what has been achieved since the first experiment. It should be used in the lowest possible concentration by using 100 mg / kg compared with industrial additives, which have increased by 200 mg / kg, to be non-toxic, natural extract, and easy to blend in food when it putting in ghee or vegetable oil we notes spread and easily melting into the product. From earlier studies it have been found that the half deathly dose value (LD_{50}) for alcoholic extract of Thyme was 6125 mg / kg of white mice weight within 24 hours [33], while the half deathly dose value (LD_{50}) for water extract of Thyme was 8125 mg / kg of birds weight within 24 hours [34].

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